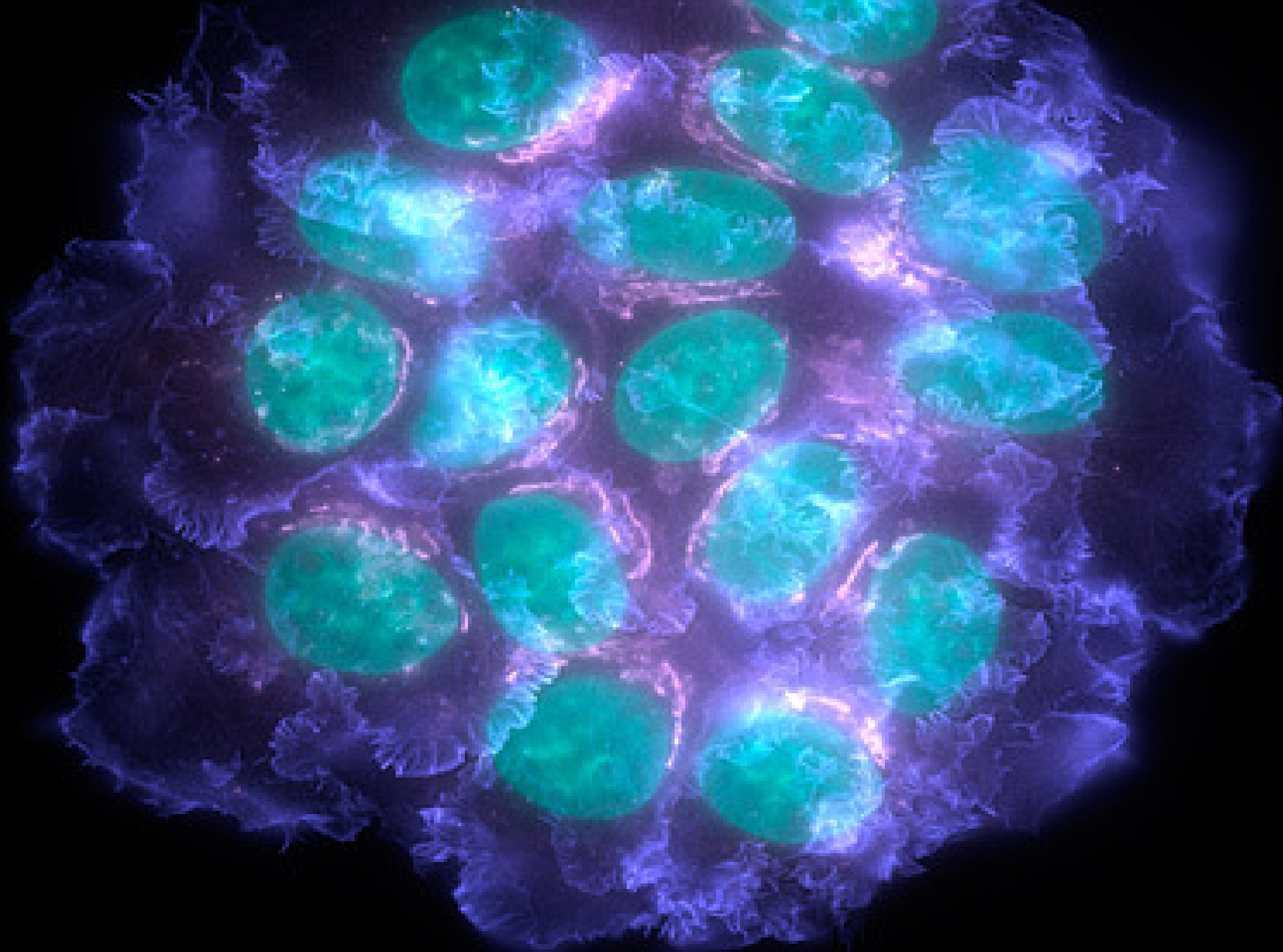


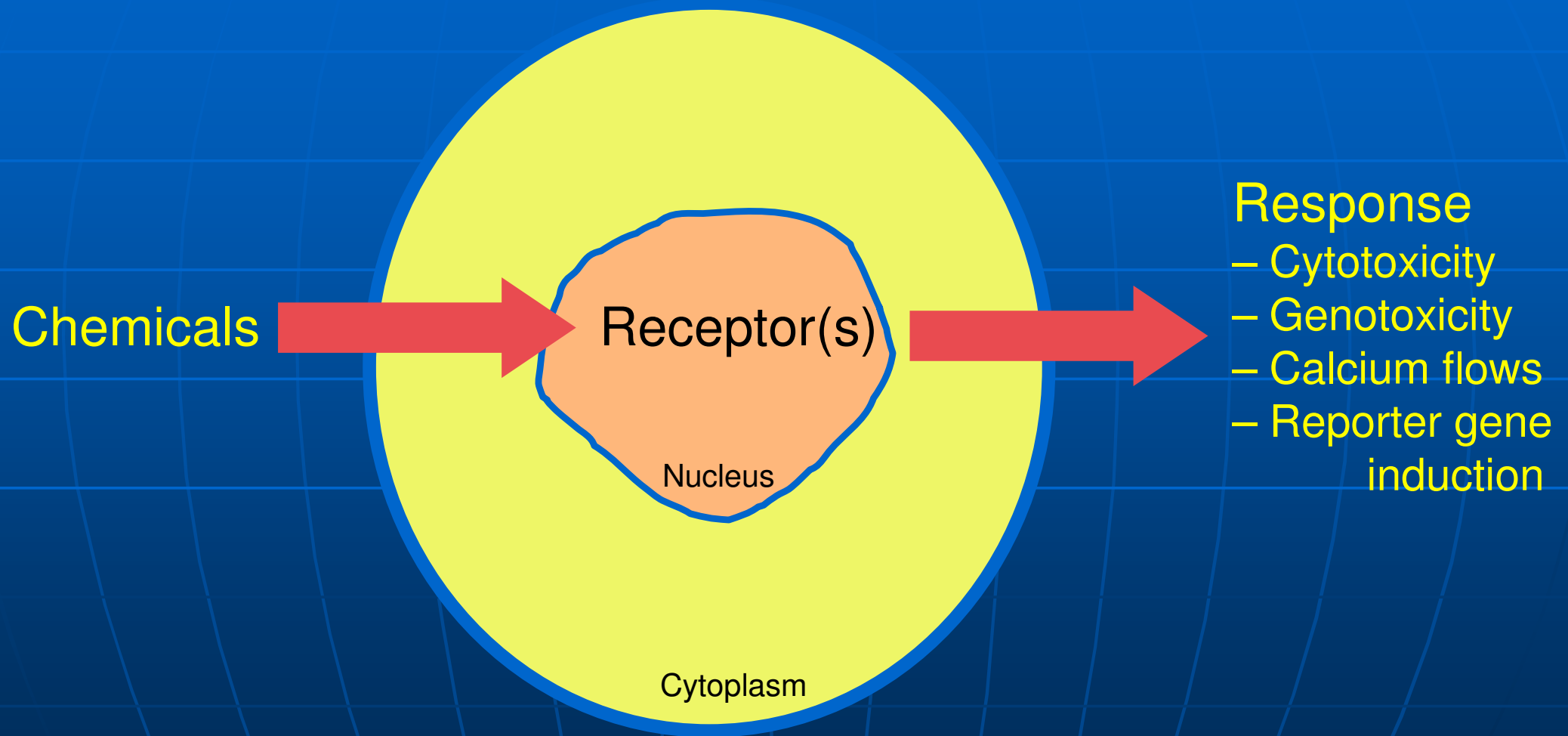
Transcriptomics in effect-based analytics

Hanspeter Naegeli

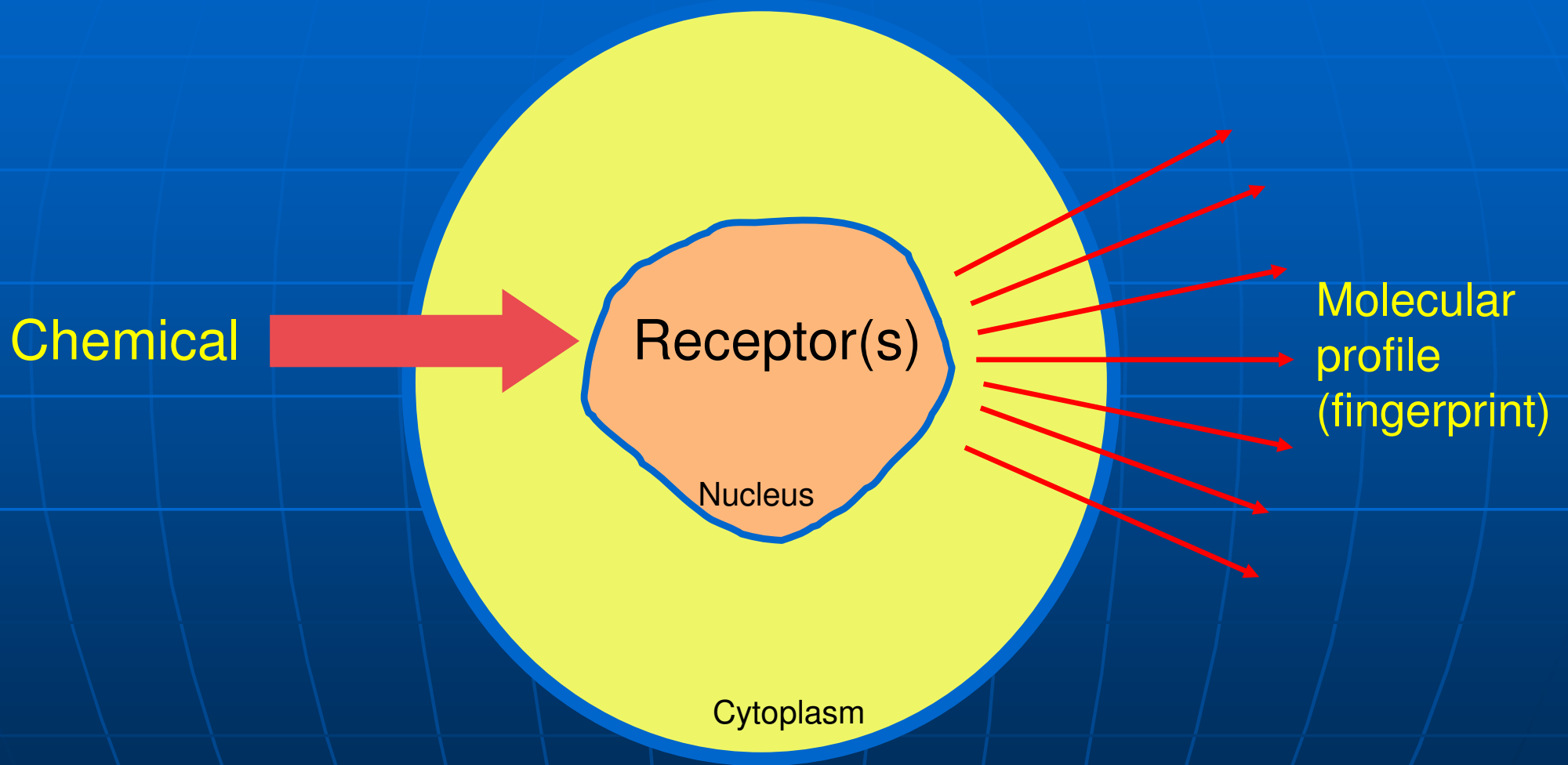
**University of Zürich
Institute of Veterinary Pharmacology & Toxicology**

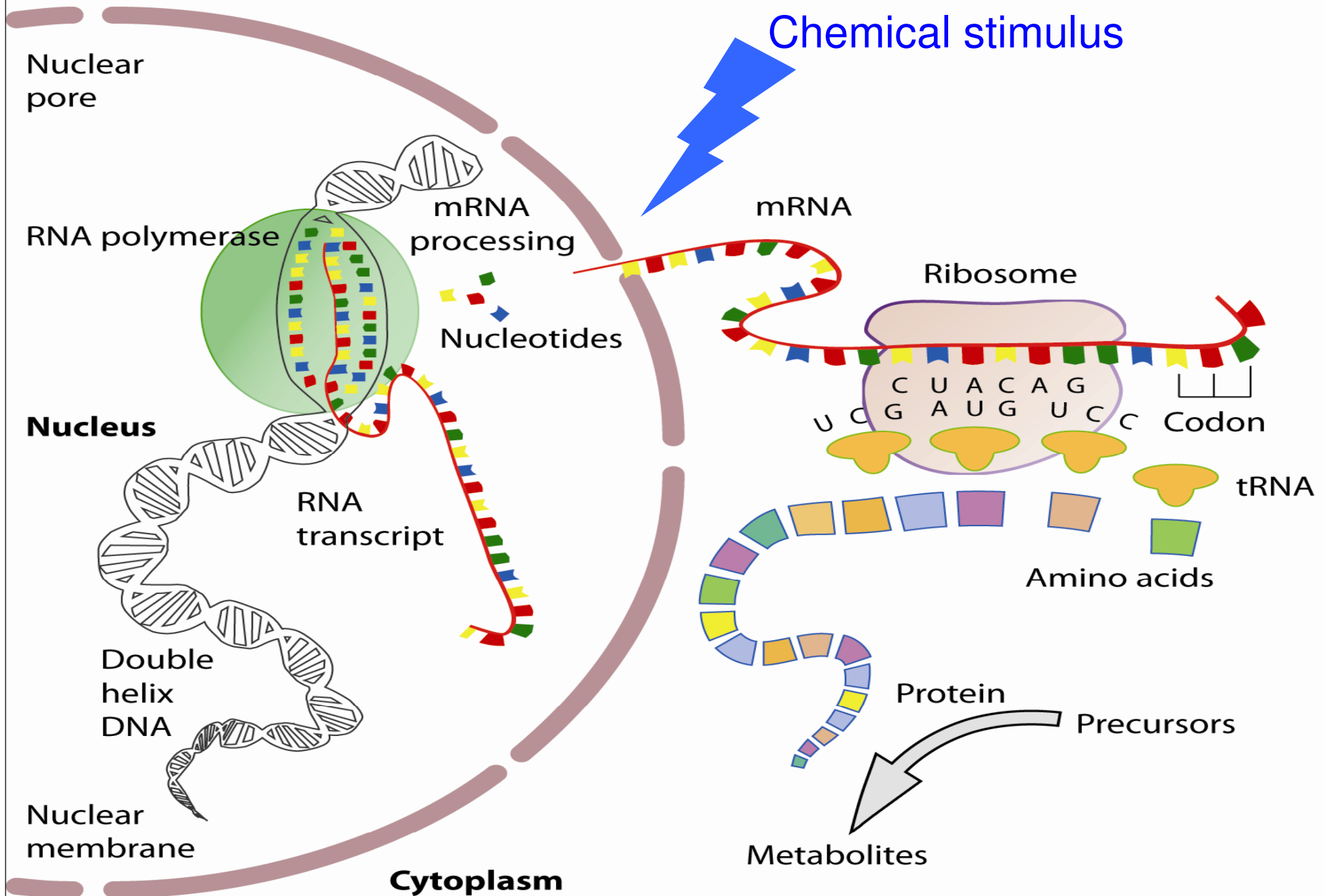


Conventional “cytosensor” assays

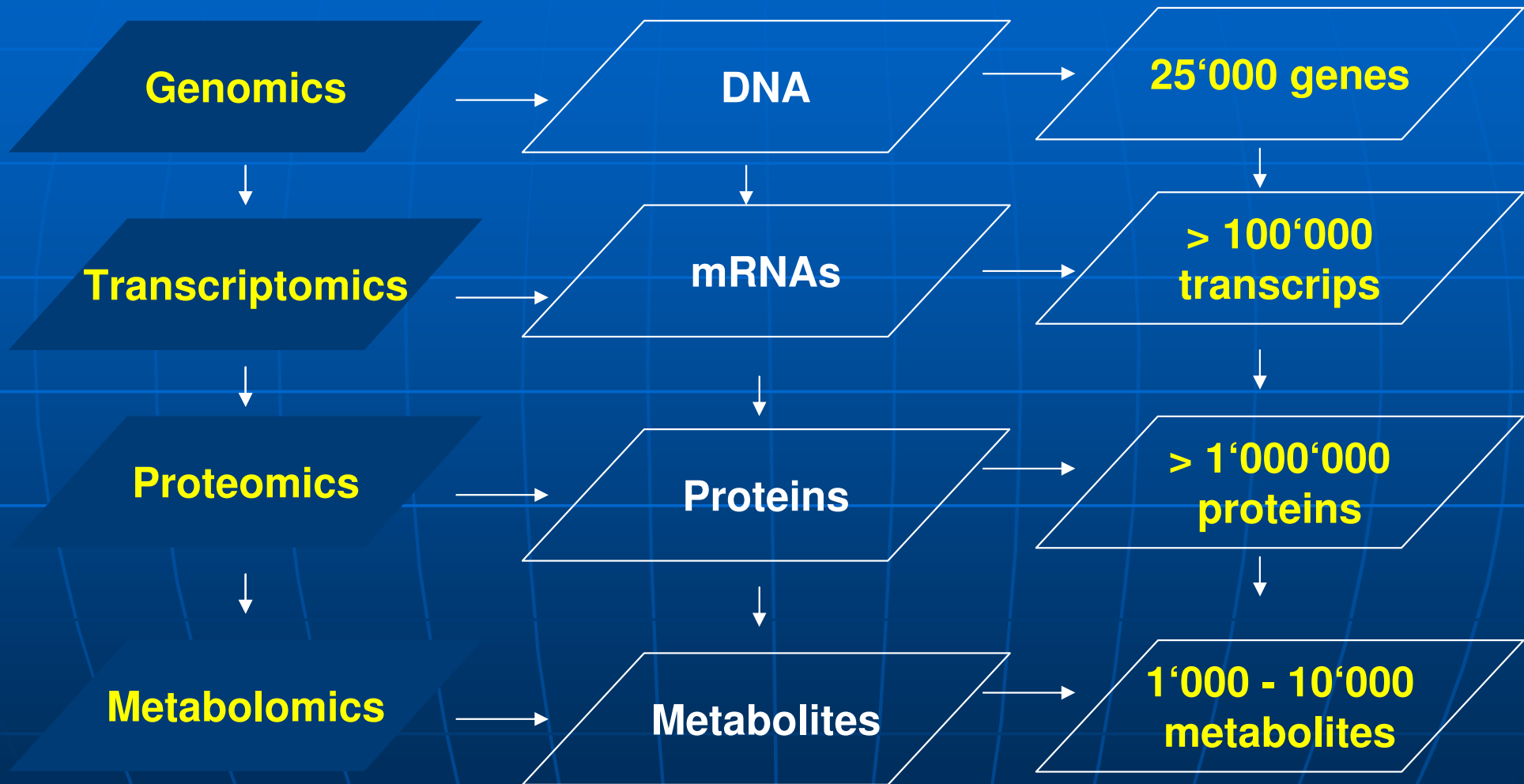


“Cytosensor fingerprinting”

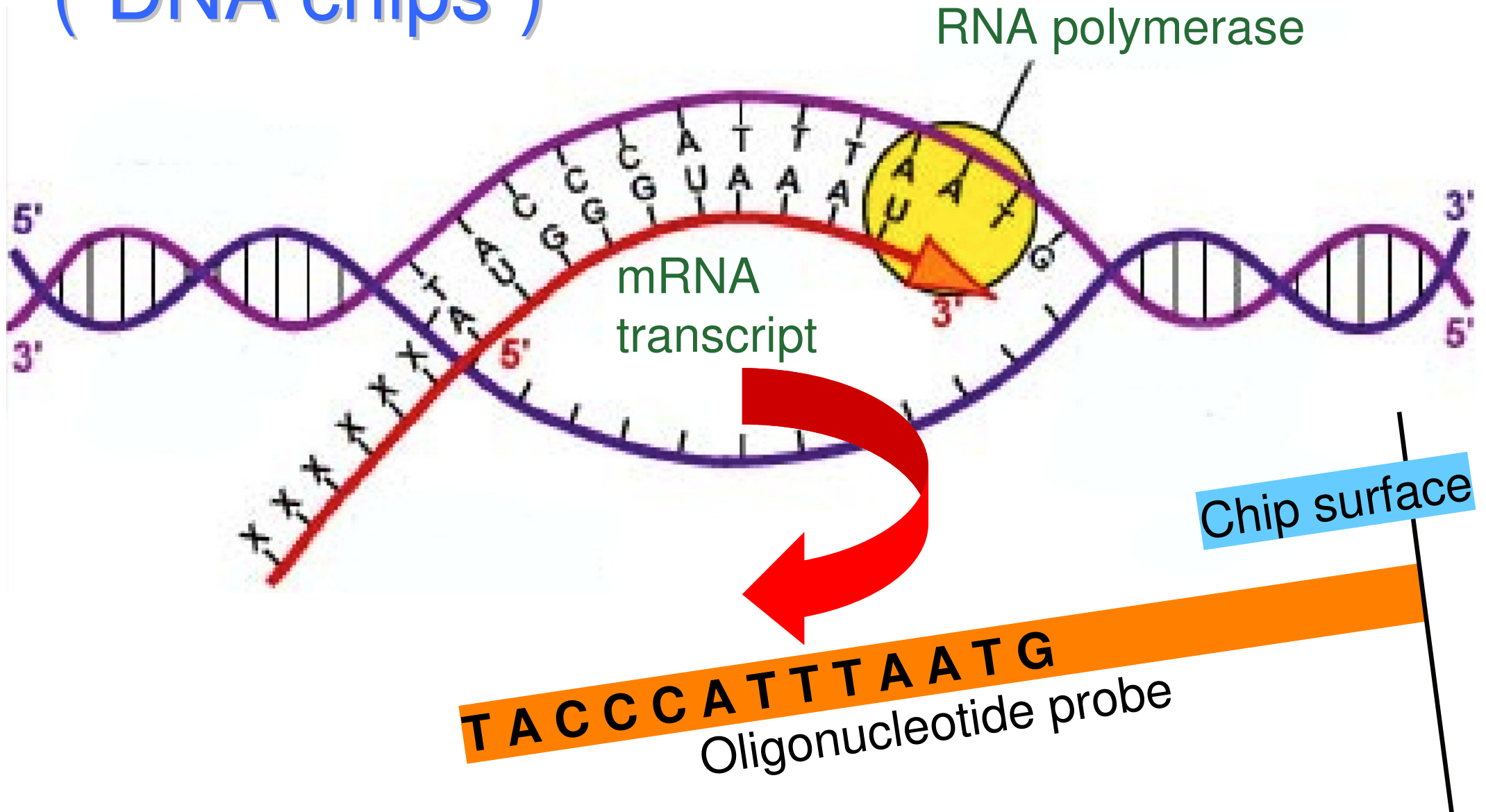




Number of endpoints



Basics of transcriptomics: microarrays ("DNA chips")





mRNA Extracted from Cell

Reverse Transcription

cDNA

Fragmented, Biotin-labeled cRNA

Biotin-labeled cRNA

Fragmentation

Transcription

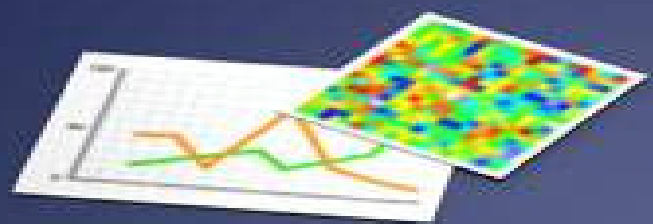
Gene-Chip Expression Array

Hybridize

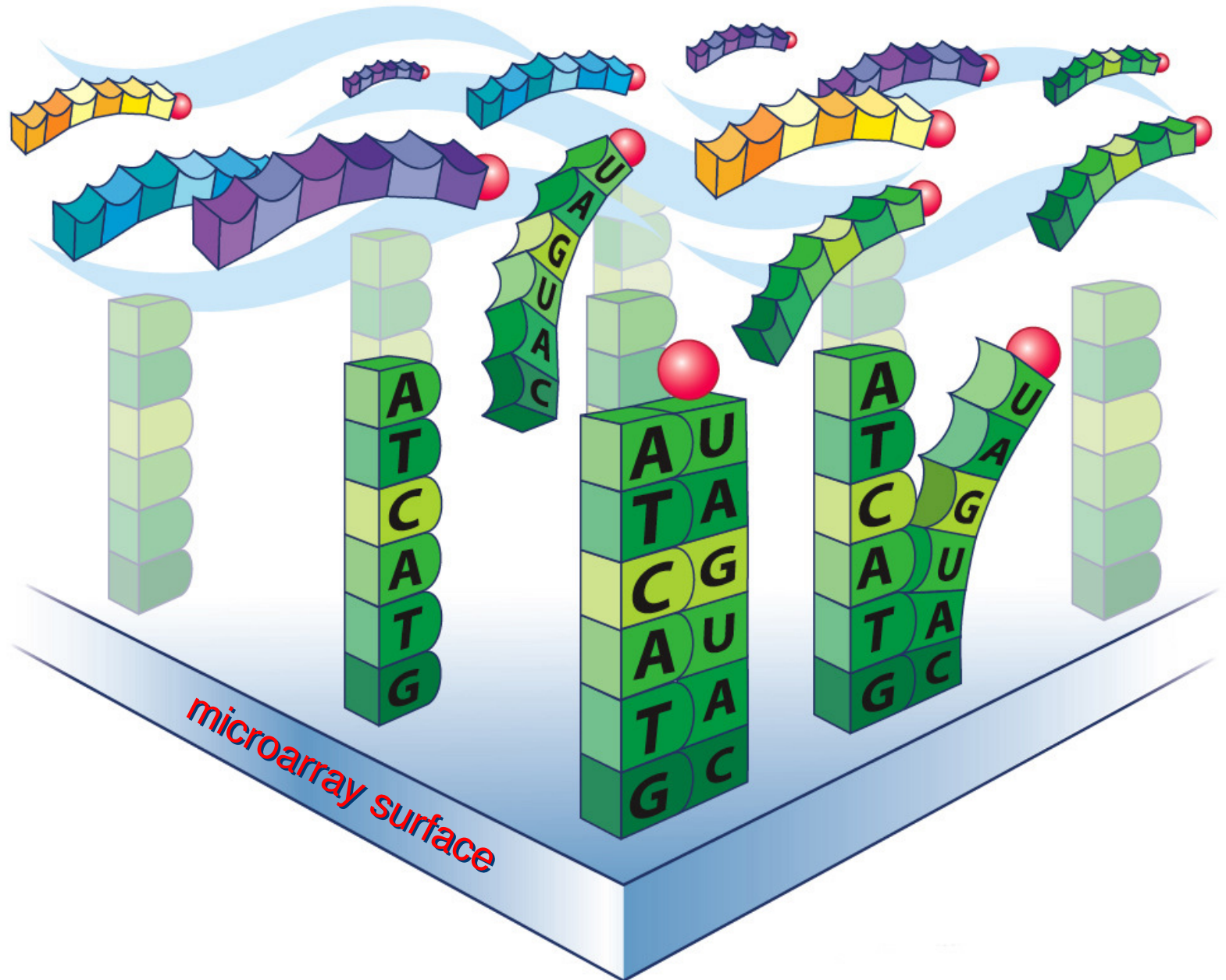
Wash and Stain

Scan and Quantitate

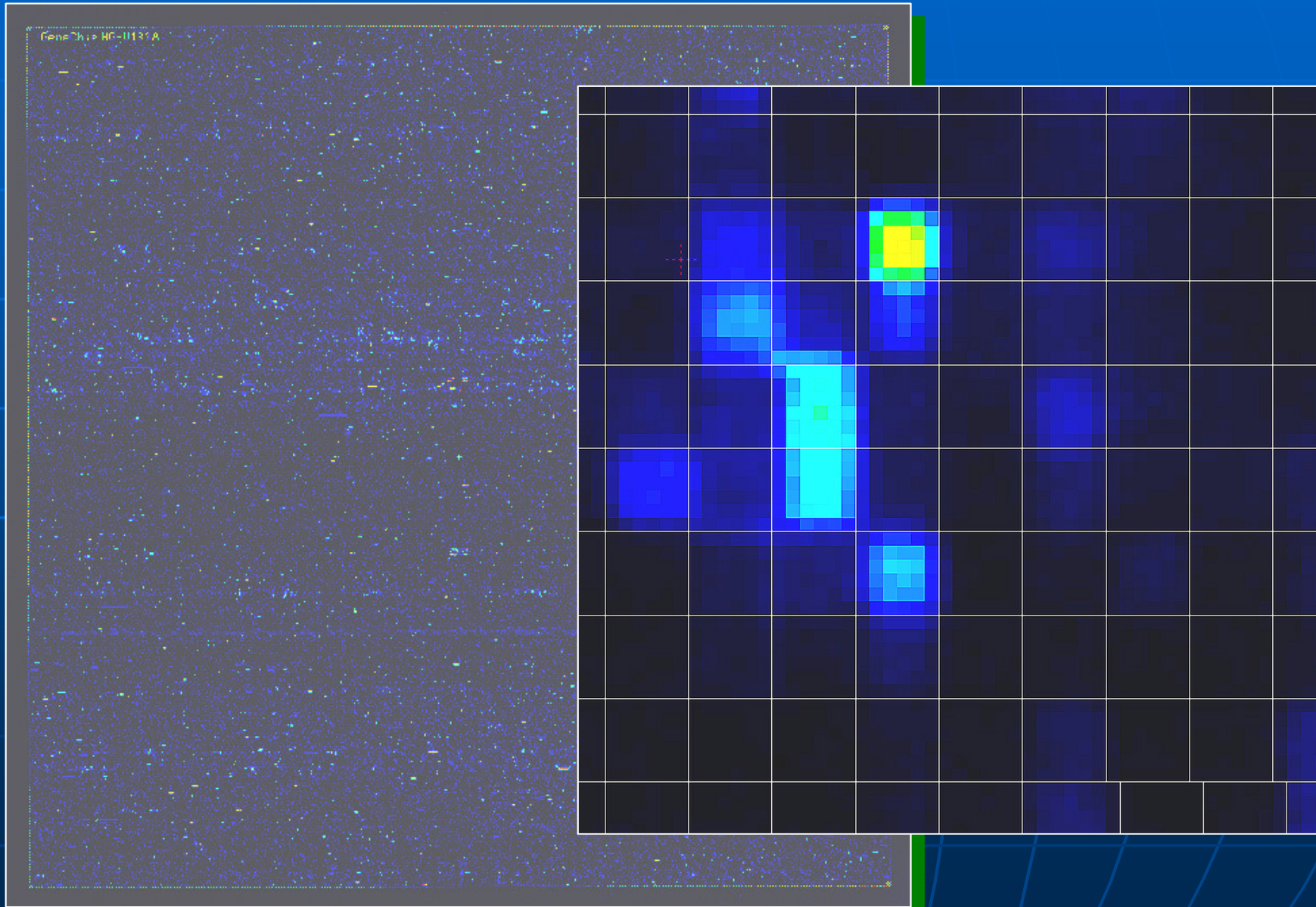
LONG



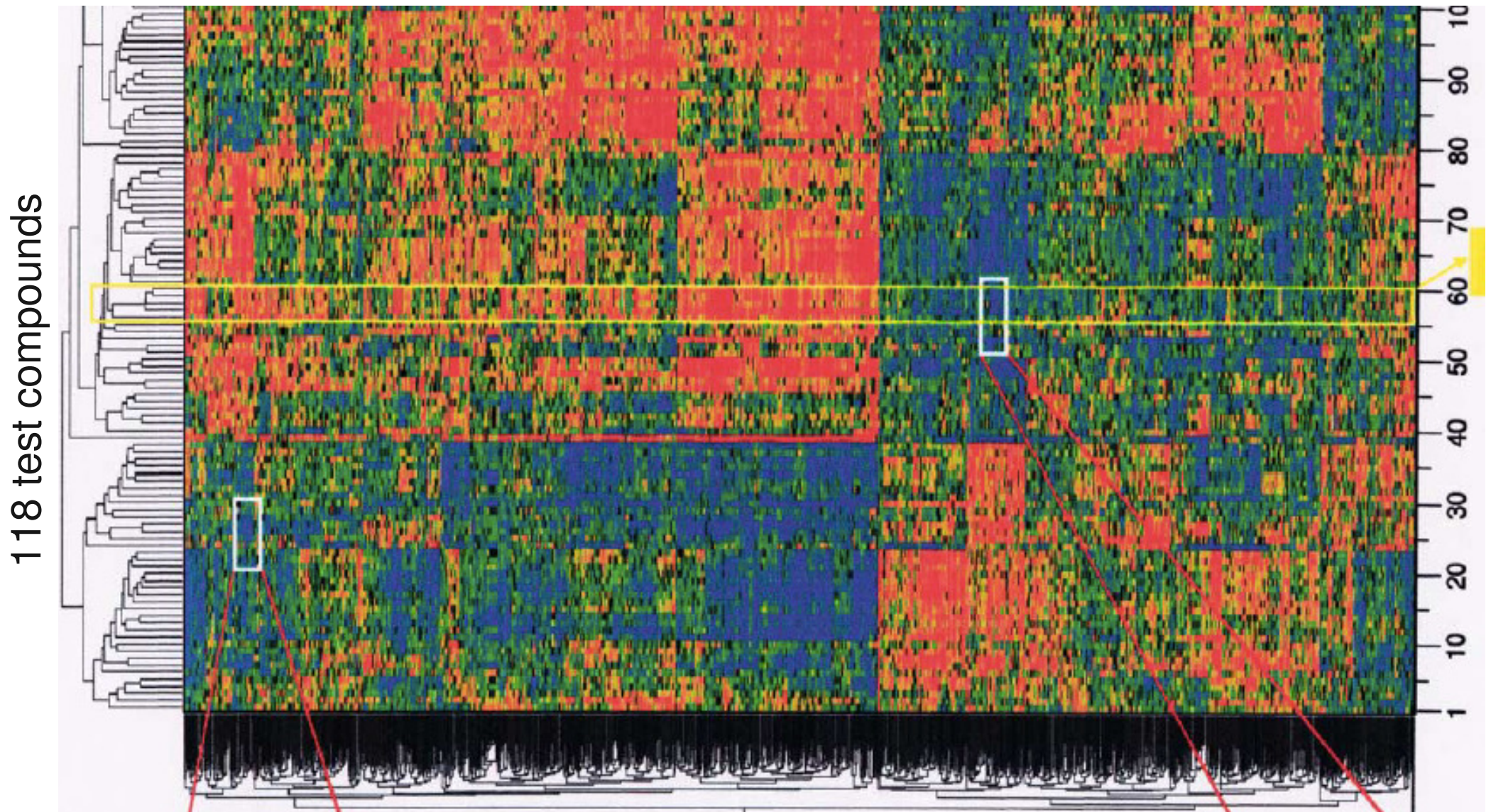
Hybridization on DNA microarrays



High-density DNA microarrays



Transcriptomic compound signatures



08/11/2012

1376 transcripts

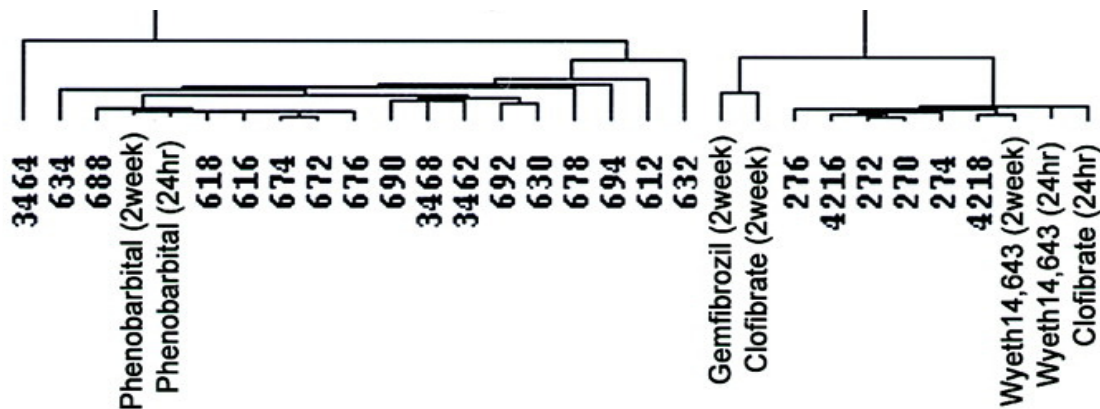
11

Weinstein & Pommier (2003) *C.R. Biologies* 326, 909-920

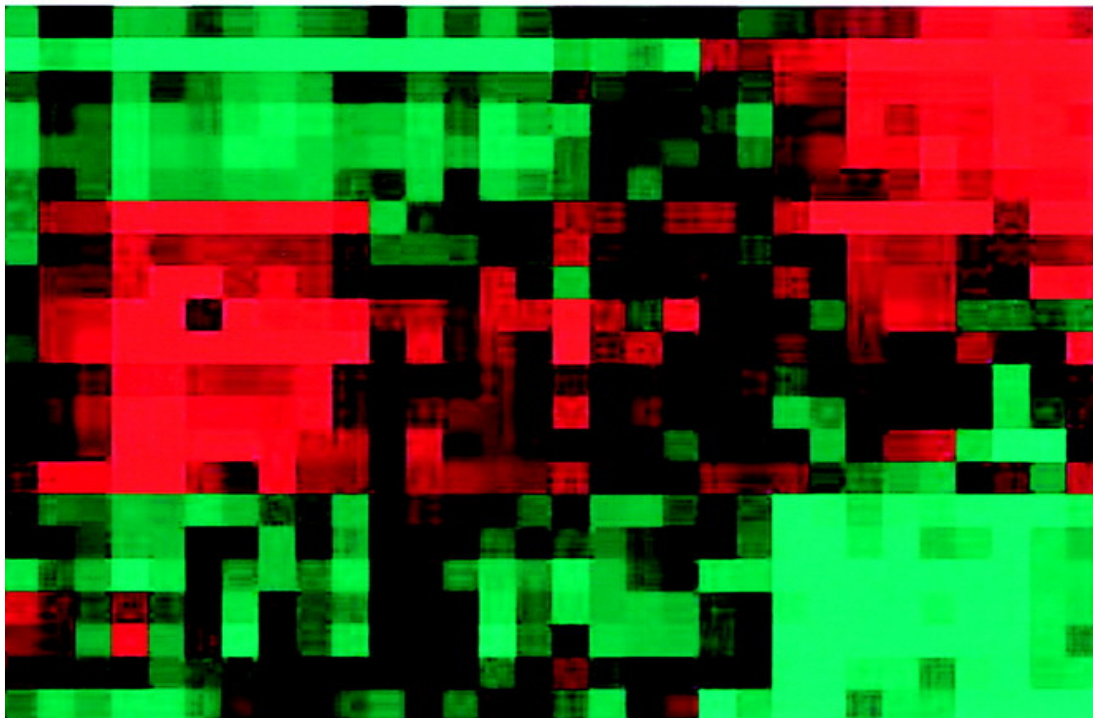
Transcriptomic hepatotoxin signatures

Liver enzyme inducers

Peroxisome proliferators

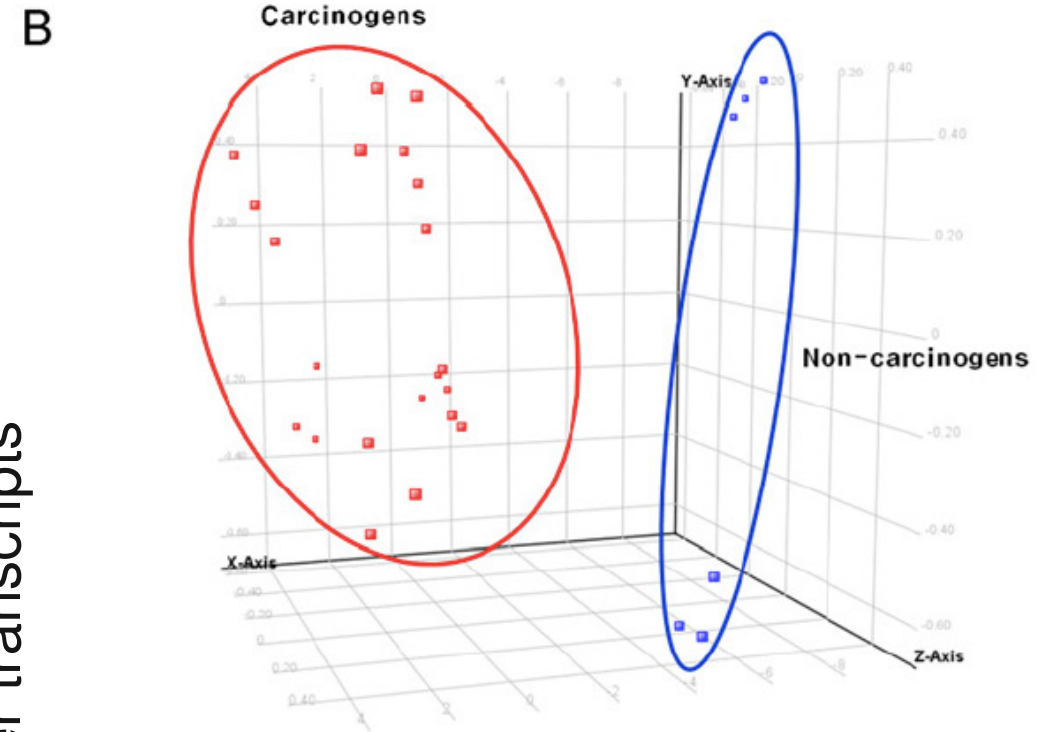
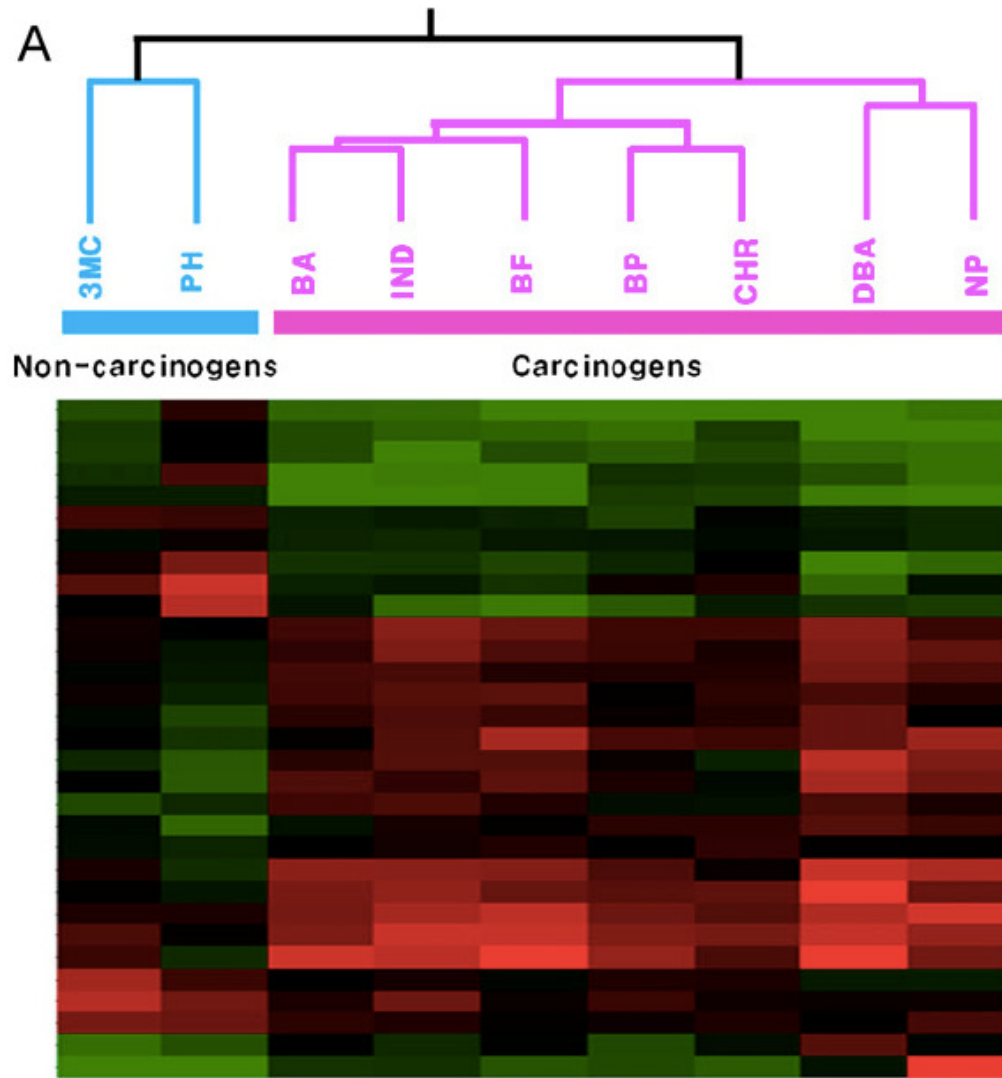


21 classifier transcripts:



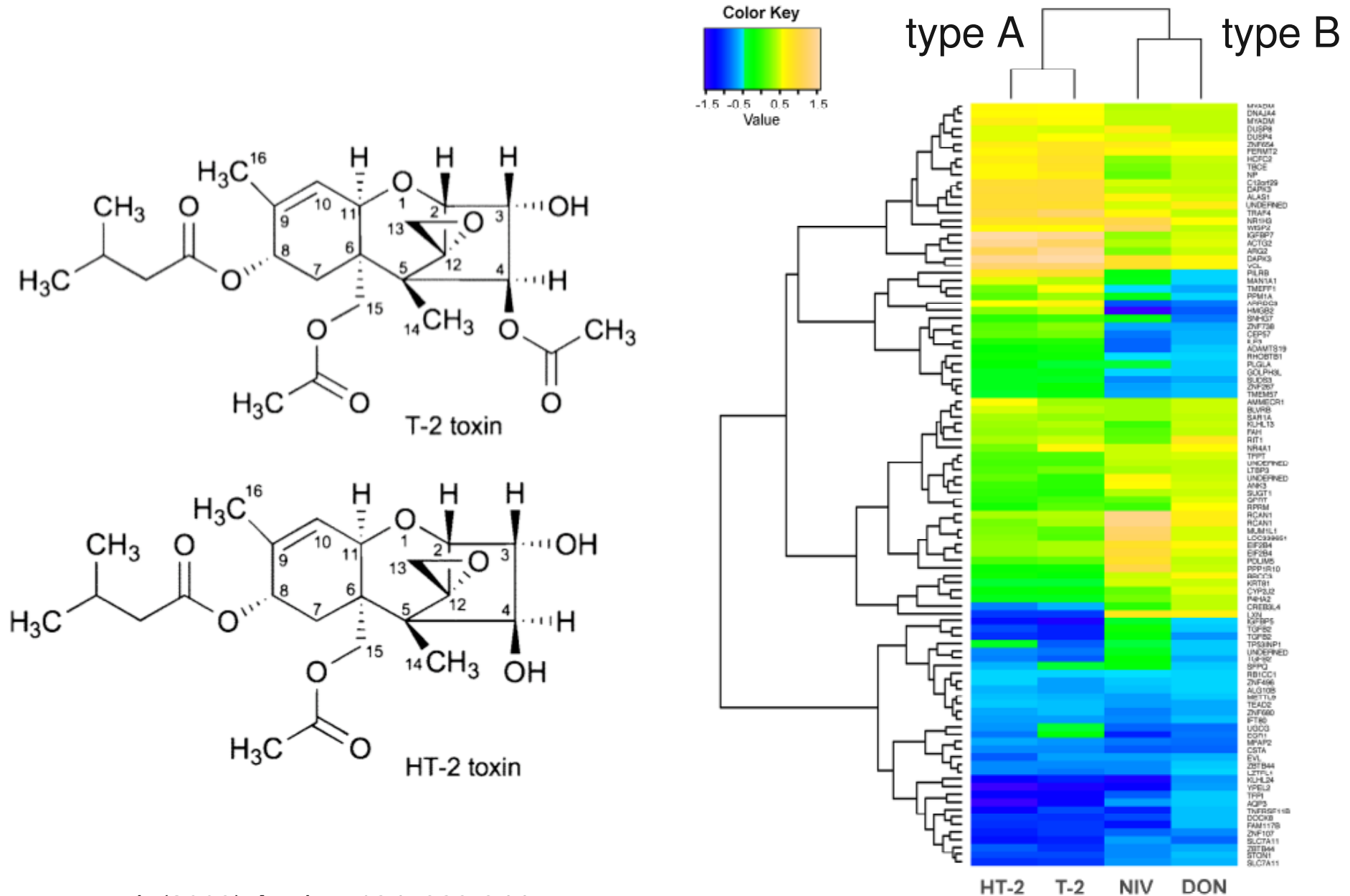
- AI070082 Mitochondrial long chain 3-ketoacyl-CoA thiolase
- AI111901 Tripeptidylpeptidase II
- AA997009 EST
- AA957359 p55CDC
- AA965078 3,2 trans-enoyl-coenzyme A isomerase
- AA964573 3-oxoacyl-CoA thiolase
- AA955106 Aldehyde dehydrogenase 1
- AI070028 UDP-glucuronosyltransferase 1
- AA899180 NADH/NADPH Diaphorase
- AA998734 Glutathione S transferase Yb2
- AA818412 Cytochrome P450 2B2
- AA924883 Phosphate cytidyltransferase 2
- AA858966 Cytochrome P450 2C6
- AA818339 Glutathione S transferase Yc
- AA859478 Aryl hydrocarbon receptor
- AA818637 Transthyretin
- AI043655 Spp-24 precursor
- AA819465 Apolipoprotein C-III
- AA819345 Parvalbumin
- AI044782 Complement component 3
- AA819595 Hydroxysteroid dehydrogenase 11 beta

Transcriptomic PAH signatures

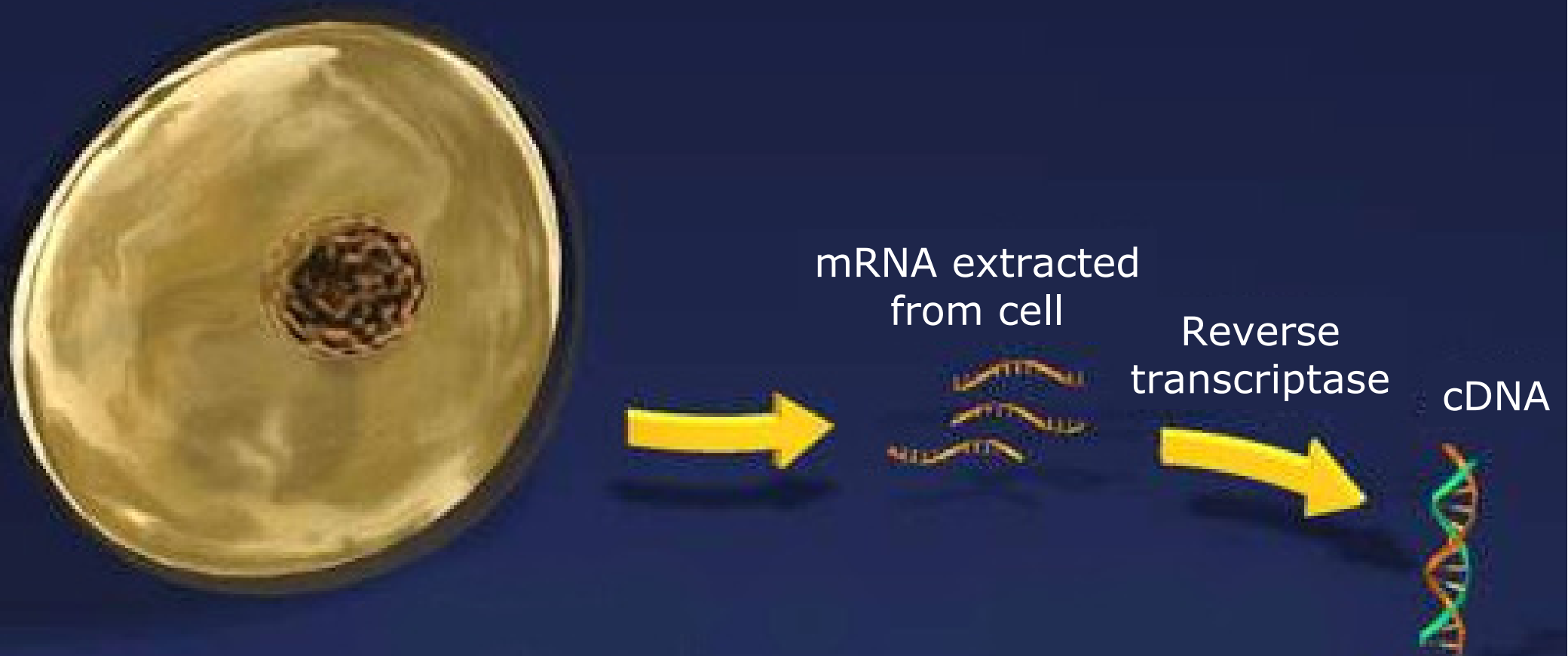


Principal component analysis
(PCA)

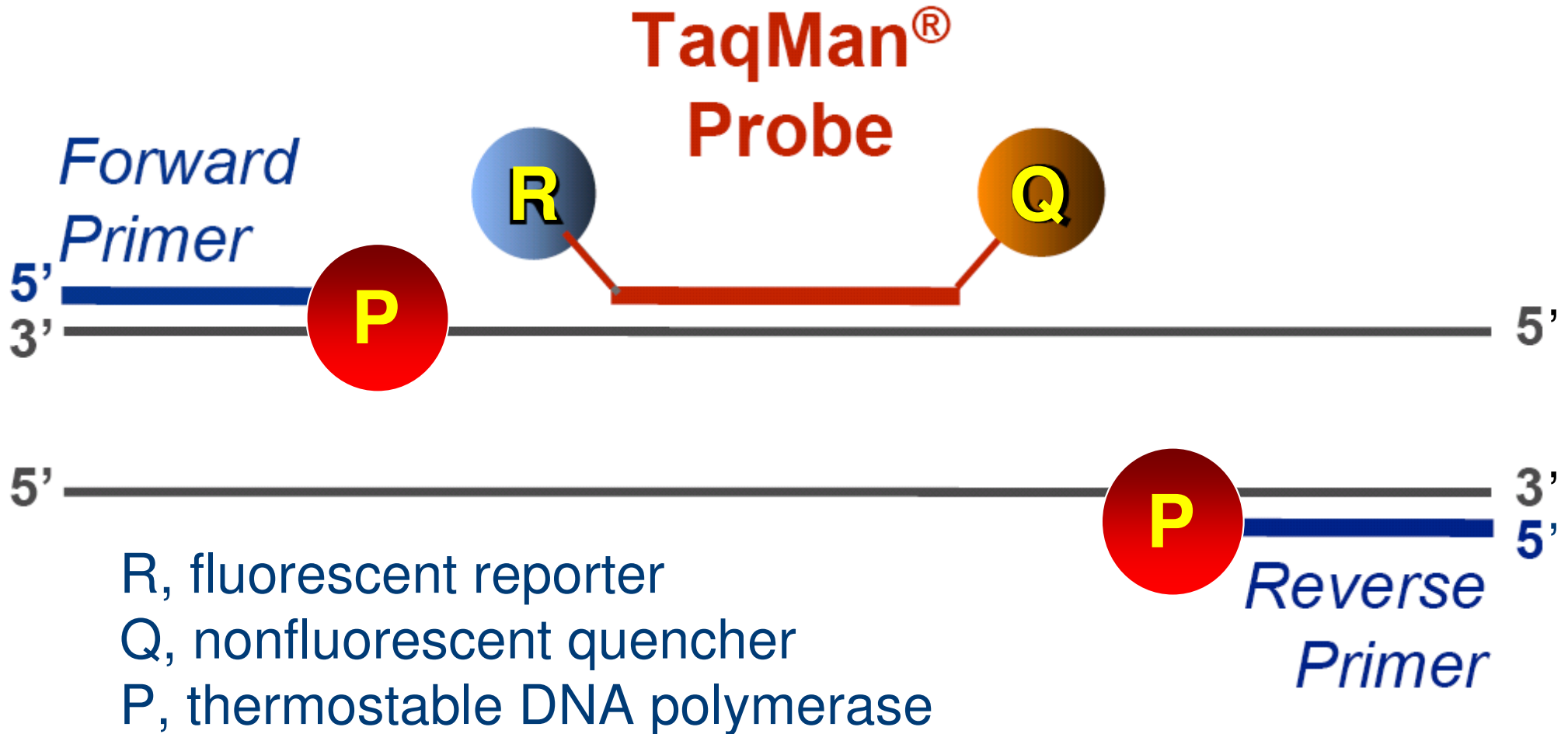
Transcriptomic trichothecene signatures



Low-density arrays

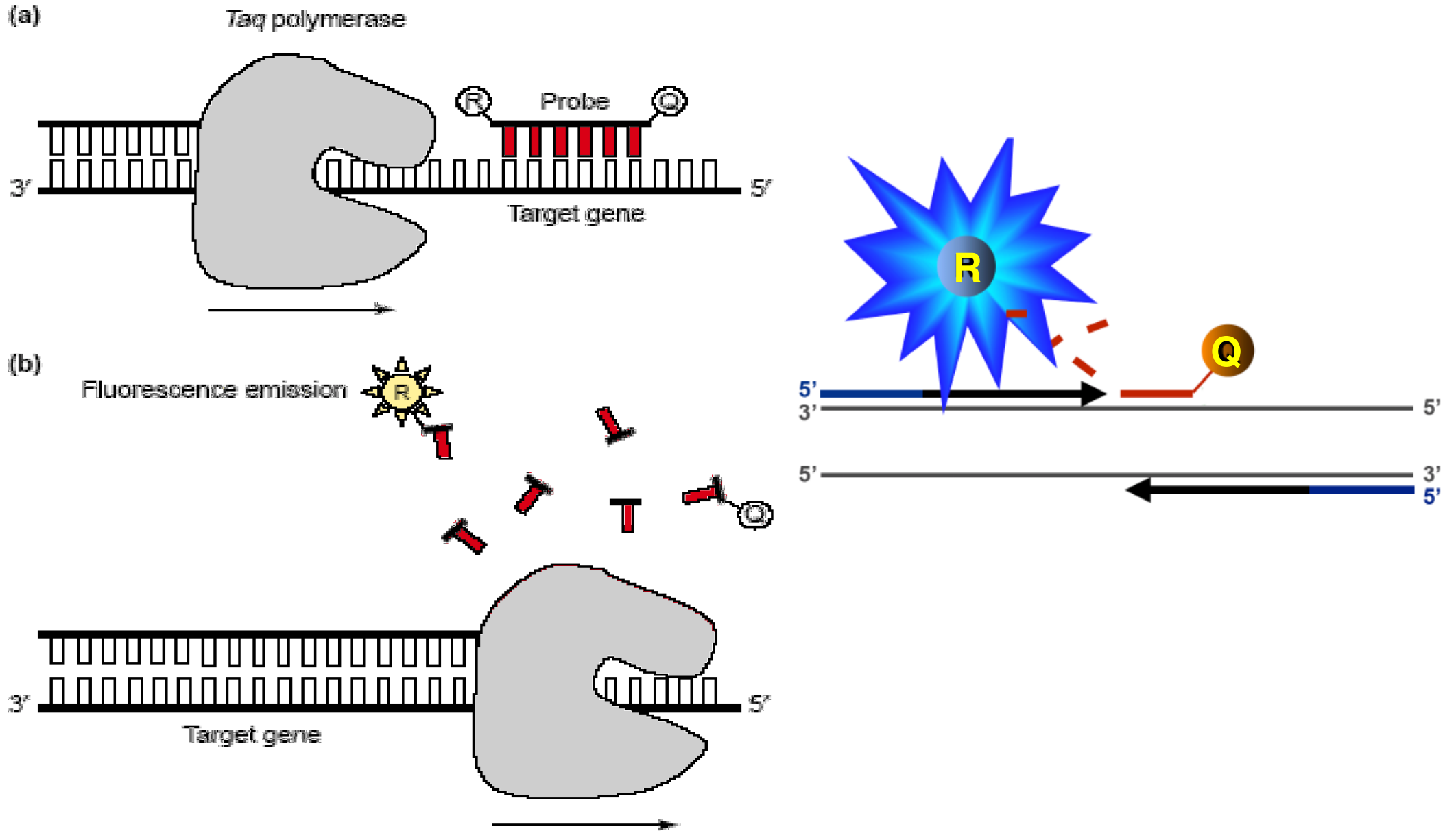


Real-time RT-PCR (1)



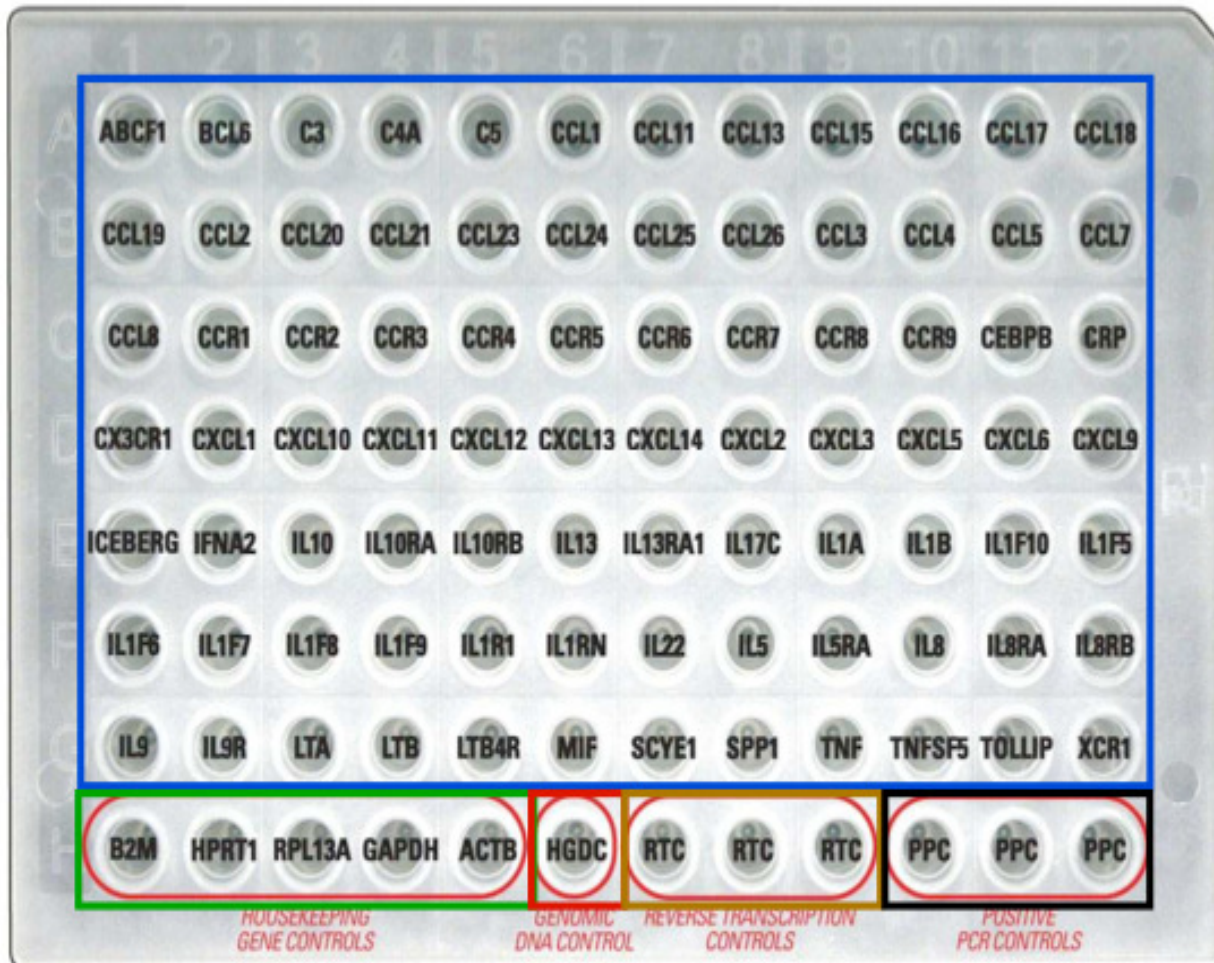
Heat denaturation → Hybridization → Primer extension → Heat denaturation

Real-time RT-PCR (2)



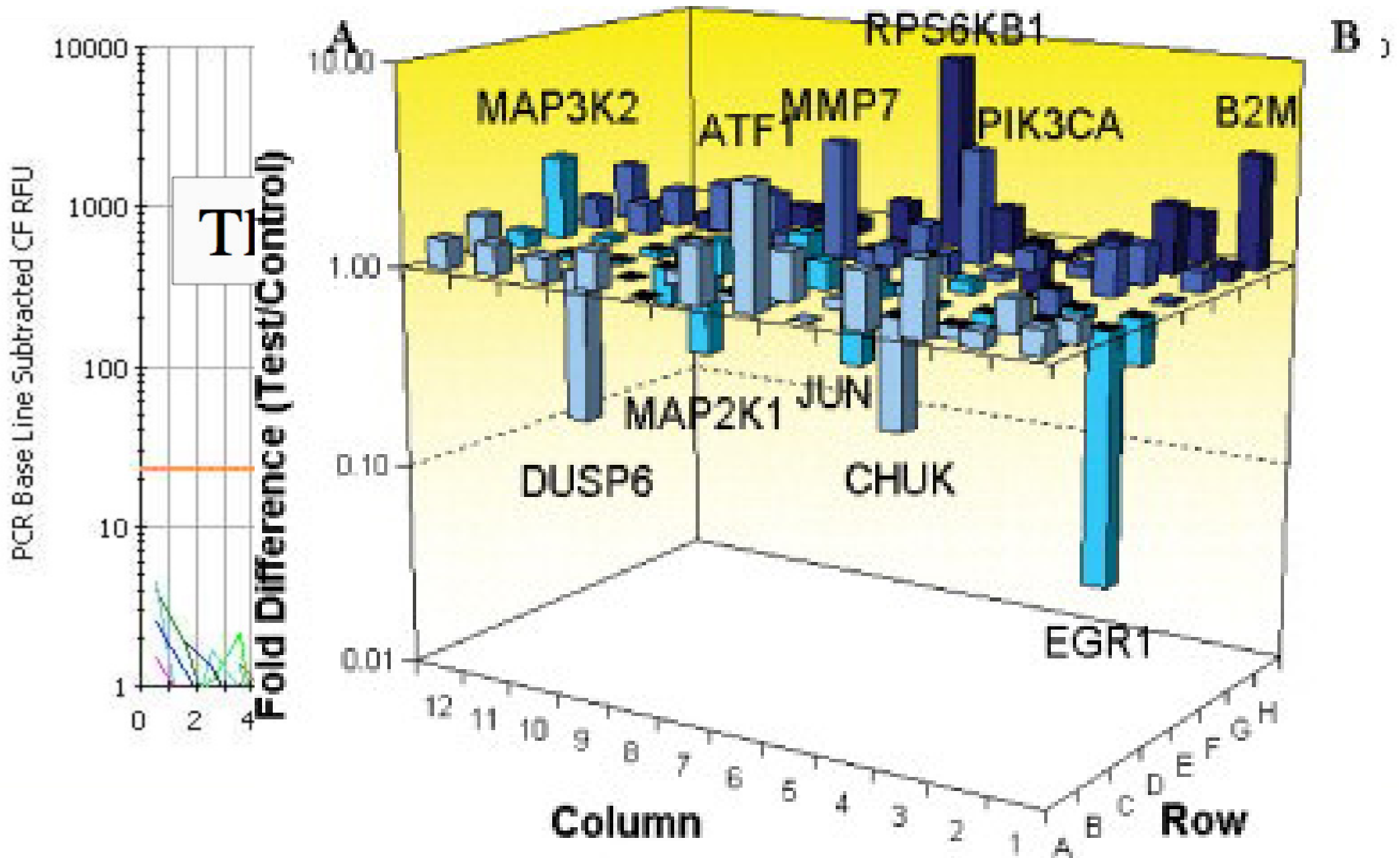
RT-PCR arrays

Human Inflammatory Cytokines & Receptors RT² Profiler PCR Array

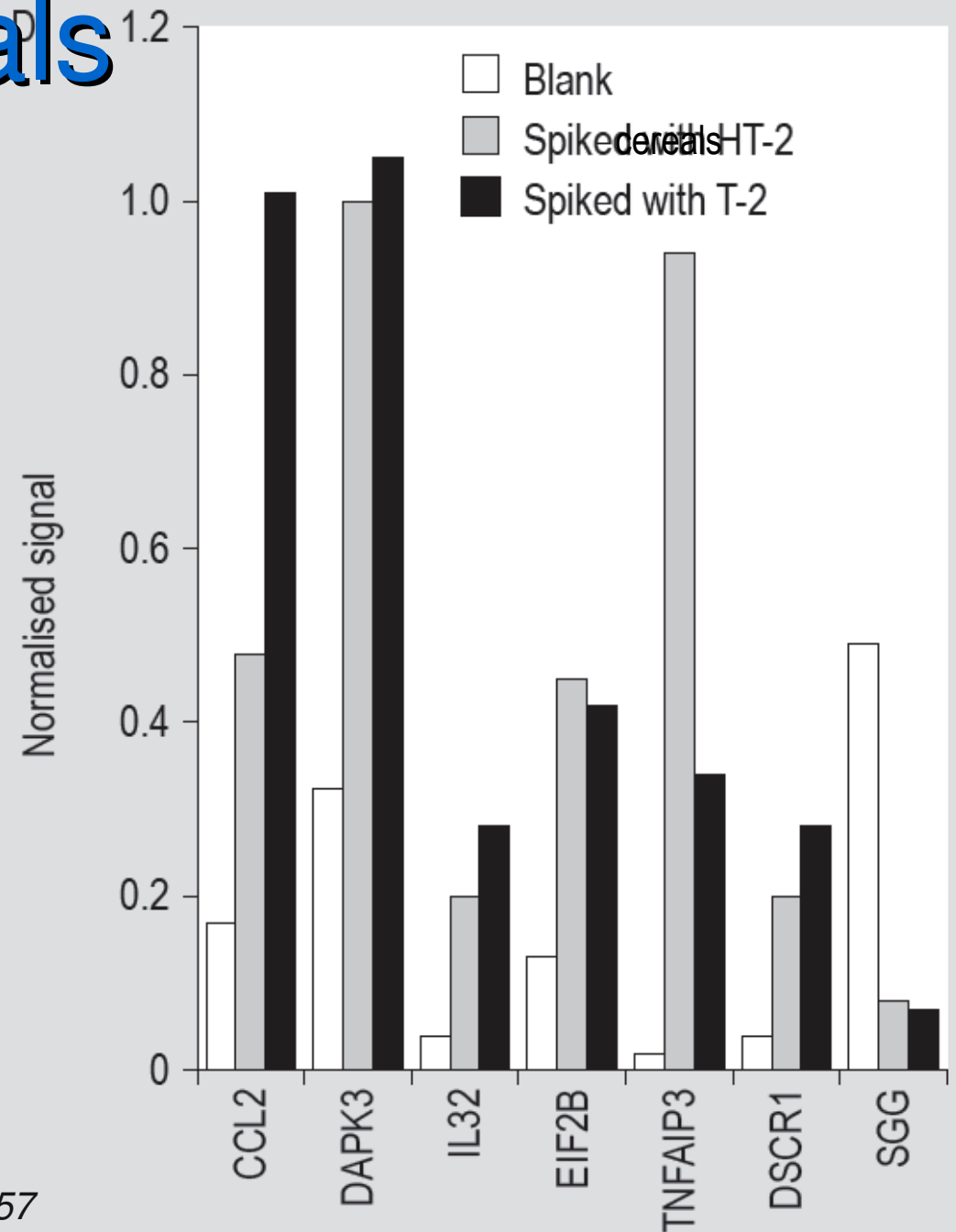
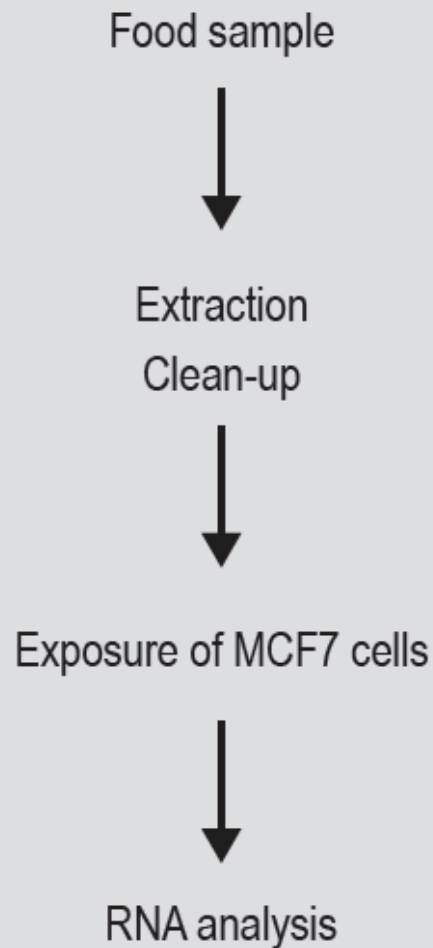


- **84 Pathway-Specific Genes of Interest**
- **5 Housekeeping Genes**
- **Genomic DNA Contamination Control**
- **Reverse Transcription Controls (RTC) n=3**
- **Positive PCR Controls (PPC) n=3**

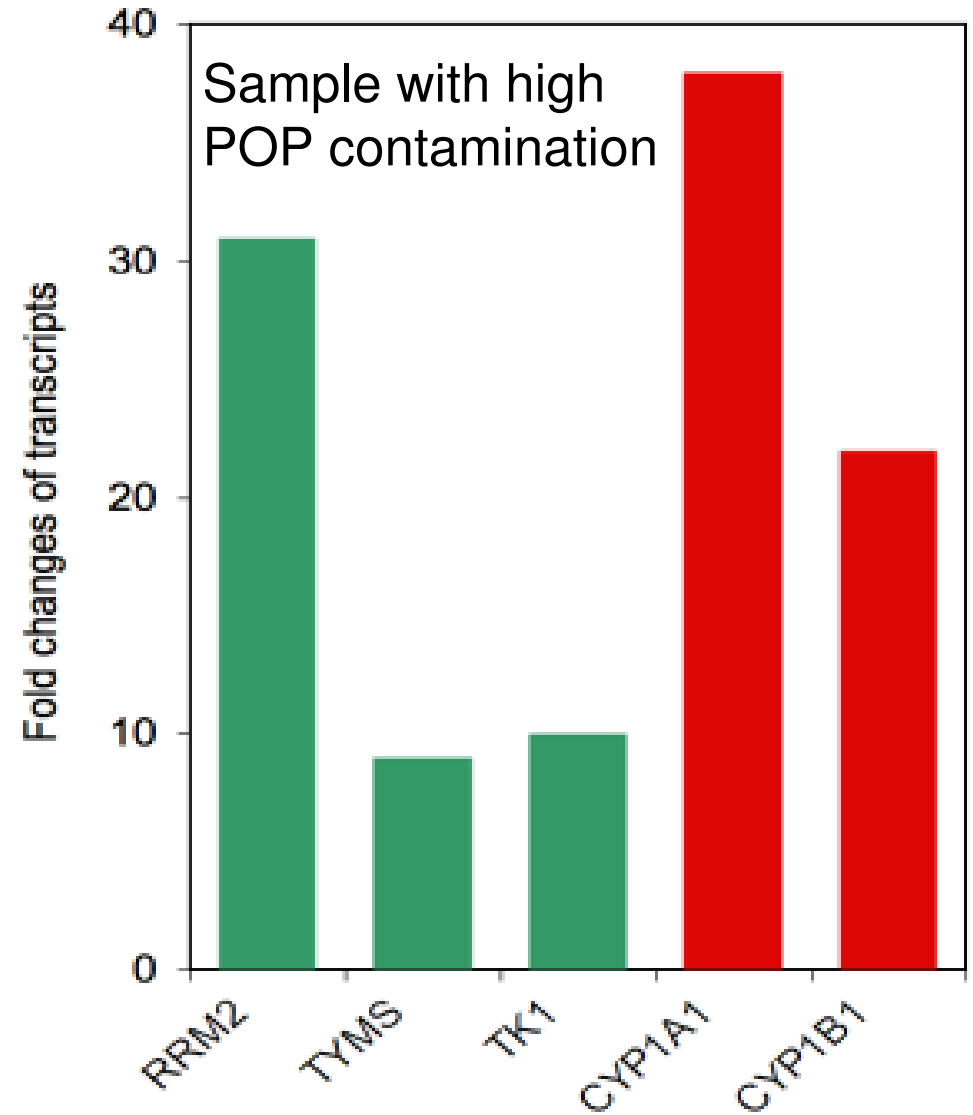
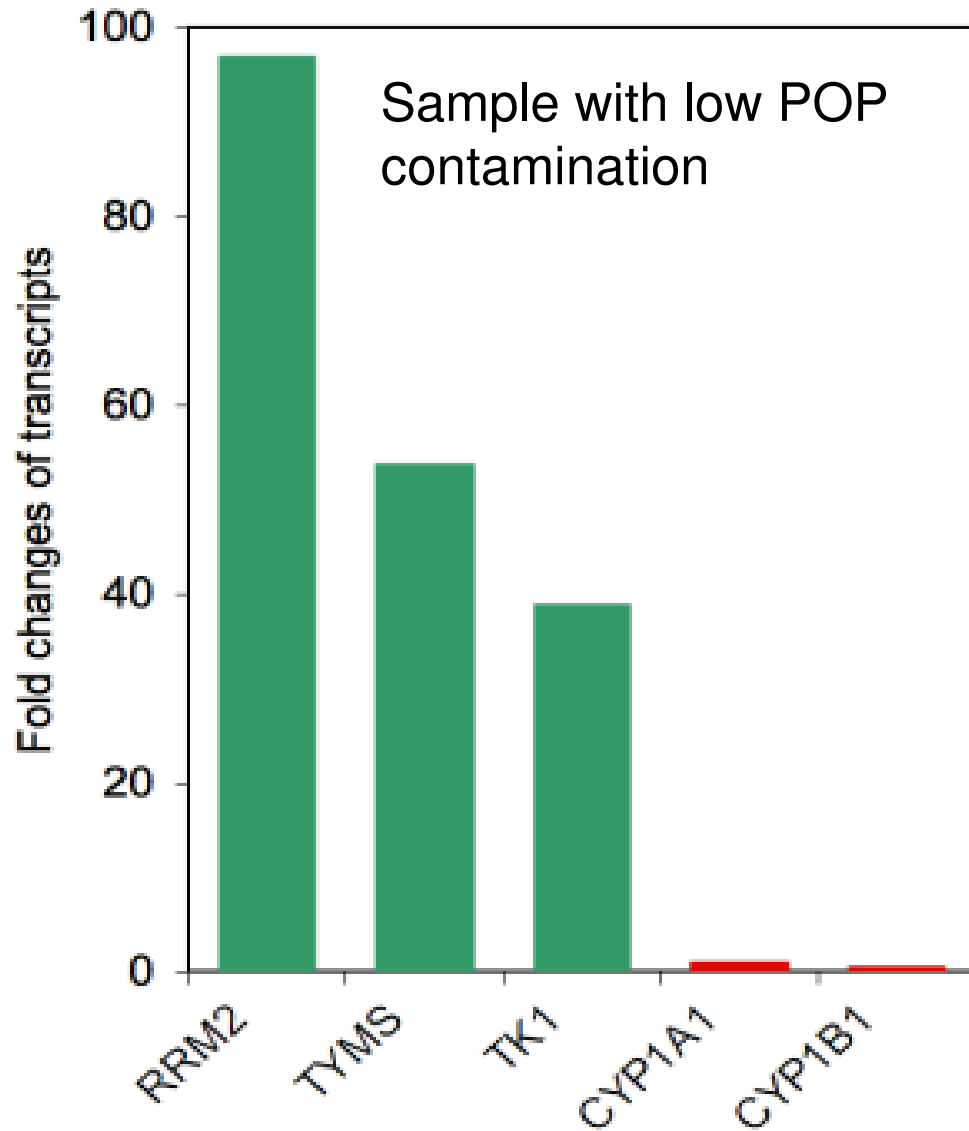
Readout of RT-PCR assays



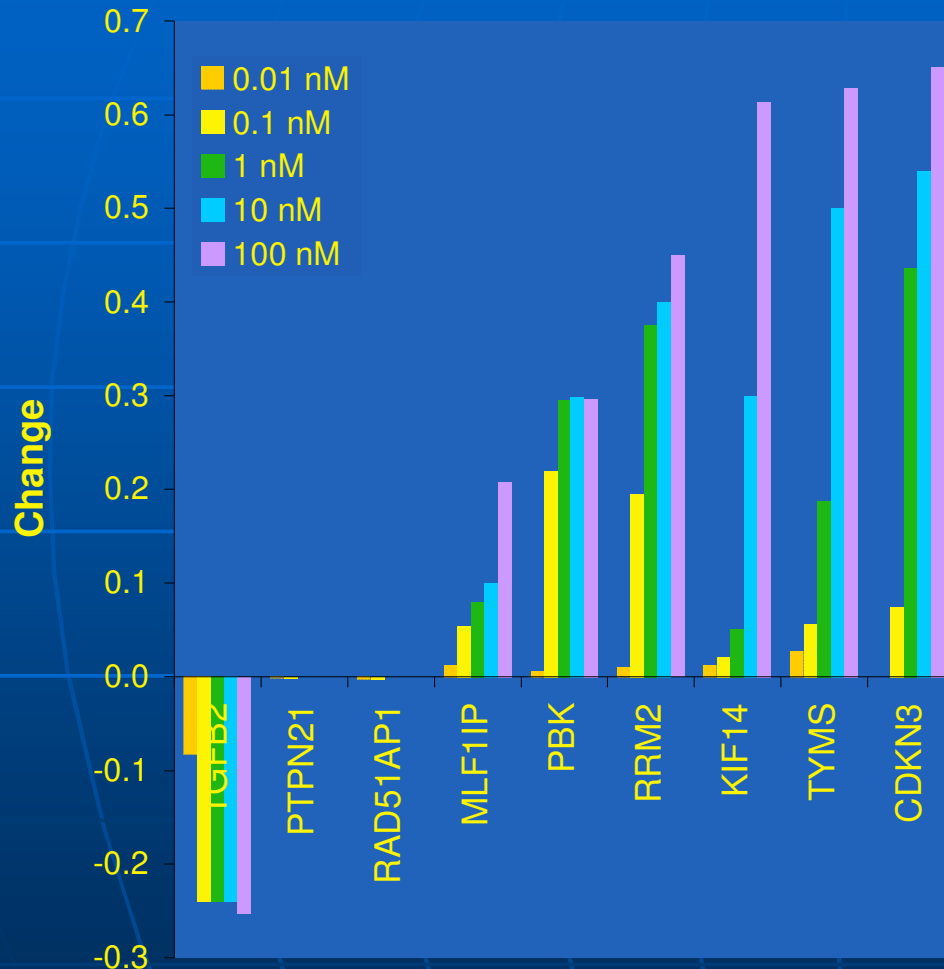
Detection of type A trichothecenes in breakfast cereals



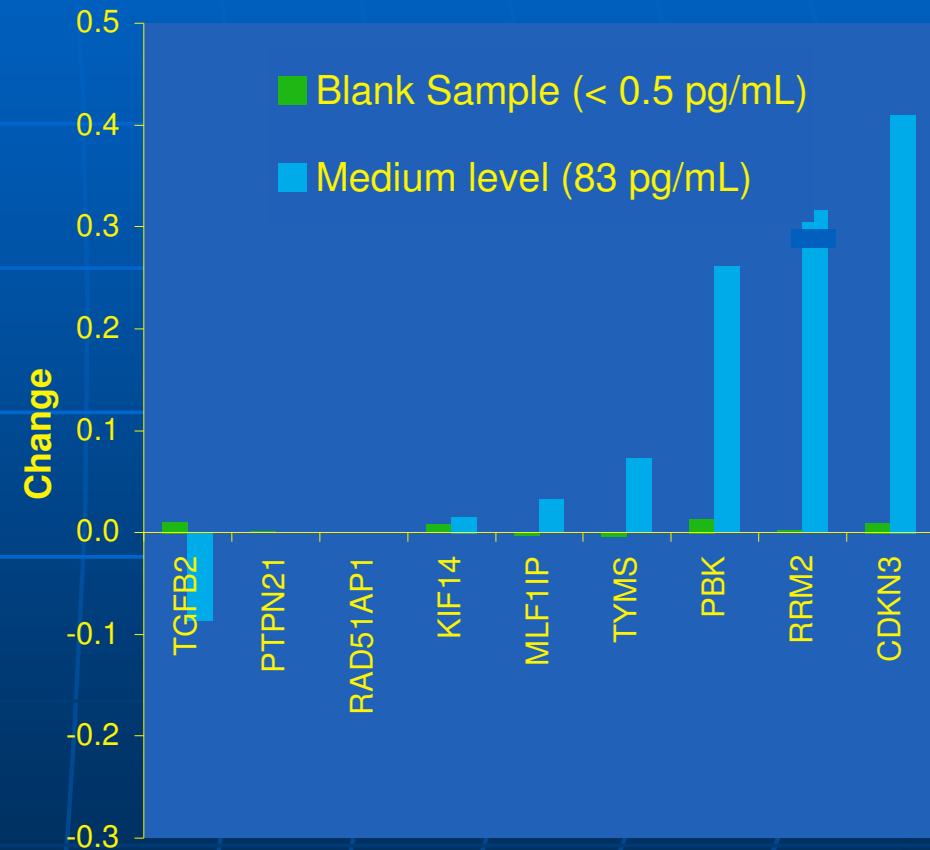
Detection of POPs in breast milk



Detection of zearalenone in maize



Zearalenone standards at five concentration levels



Maize sample contaminated with zearalenone (certified reference material)

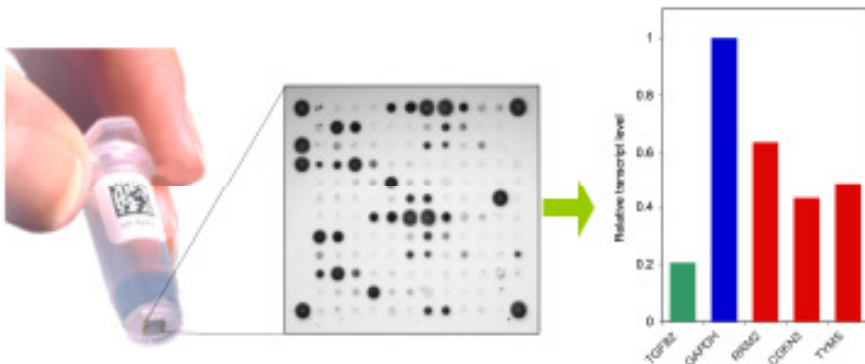
Conclusion: Food analysis by cytosensor fingerprinting

Food extract

Cultured human MCF7 cells

Changes of RNA transcripts

- Multi-endpoint strategy based on molecular fingerprinting
- Advantages in comparison to conventional bioassays:
 - Improved sensitivity/selectivity
 - Biologically relevant endpoint in a toxicologically significant target
 - Simultaneous detection of multiple contaminant groups



Future trends

- Increased responsiveness of cytosensor by integration of additional receptors
- Miniaturization, automation
- Three-dimensional cell culture systems
- Use of induced pluripotent stem cells